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The Glutamic–Aspartic Transaminase of Extracts of Vegetative Cells and of Spores of *Bacillus megatherium*

Levinson and Sevag (1, 2) have reported on the stimulation of germination of the spores of *Bacillus megatherium* by manganese and on the occurrence of a manganese-stimulated proteolytic enzyme in extracts of *B. megatherium* spores. Contrary to the reports of Hardwick and Foster (3), we have now demonstrated that spore extracts also possess a strong glutamic–aspartic transaminase. In these experiments, the system α-ketoglutarate + aspartate $\rightleftarrows$ glutamate + oxalacetate was investigated.

Vegetative cells of *B. megatherium* QM B1551 were harvested after 12 hr. in the liver medium of Foster and Heiligman (4), and the spores were harvested after 5 days in the same medium. Both spores and vegetative cells were washed twice in phosphate buffer and at least three times in cold distilled water, before being dried from the frozen state.

Extracts were prepared by grinding 500 mg. of the lyophilized cells with 2.0 g. of powdered Pyrex glass in a Potter mill at a temperature of 5–10°C., together

with enough 0.1 *M* phosphate buffer at pH 7.4 to make a thick paste. After 30 min. of intermittent grinding, the paste was taken up in 10 ml. of the 0.1 *M* phosphate buffer, and centrifuged for 20 min. at approximately 3000 × *g* at a temperature of approximately 5°C. The microscopically cell-free supernatant was used as the source of enzyme. Of the total spore nitrogen, 18.2%, and of the total vegetative cell nitrogen, 21.2% were extracted in the grinding. In the typical experiment reported below, the spore extract had 0.818 mg. N/ml., of which 0.421 mg. was protein N (trichloroacetic acid precipitation), and the vegetative cell extract had a total nitrogen concentration of 0.967 mg./ml., of which 0.652 mg. was protein N.

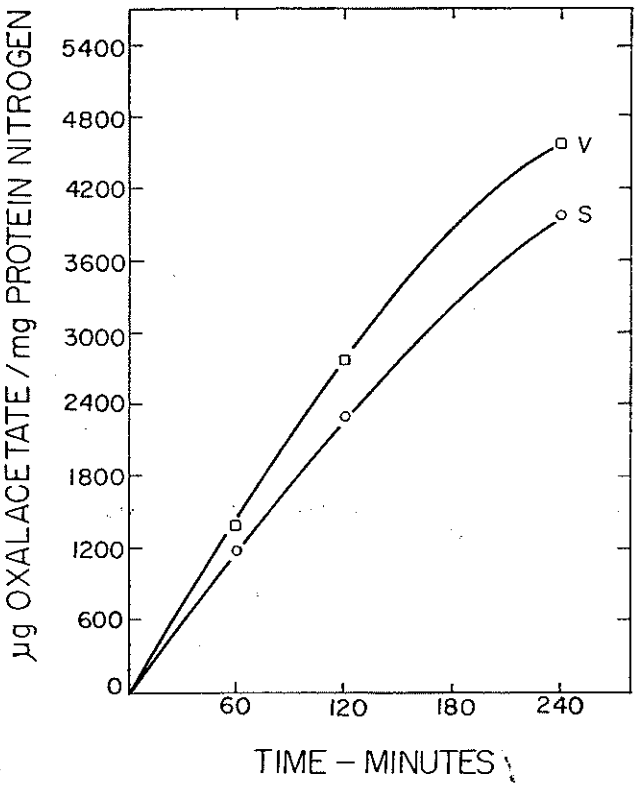

FIG. 1. Oxalacetate produced from α-ketoglutarate and aspartate via glutamic–aspartic transaminase of spores and vegetative cells of *B. megatherium*. Oxalacetate measured as pyruvate according to the method of Tonhazy *et al.* (5). Curve V, vegetative cell extract; curve S, spore extract.

The reaction systems contained: 0.2 ml. of 0.5 *M* phosphate buffer, pH 7.4; 0.1 ml. pyridoxal phosphate, acid ester calcium salt (Merck and Company) containing 20 μg.; 0.4 ml. L-aspartic acid, 0.0625 *M*, adjusted with KOH to pH 7.2; 0.1 ml. α-ketoglutaric acid, 0.5 *M*, adjusted with KOH to pH 7.2; and 0.2 ml. extract. The reaction was stopped at the desired intervals by the addition of 0.1 ml. of 100% trichloroacetic acid.

The oxalacetate formed in the reaction was measured as pyruvate according to the method of Tonhazy *et al.* (5). Glutamate produced was detected by paper-partition chromatography (descending method) with 75% isopropyl alcohol–5% acetic acid as the solvent. Before the addition of trichloroacetic acid to the tubes containing the reaction mixtures, 10 μl. was applied to Whatman No. 1 paper with a Kirk micropipet at appropriate time intervals. Ninhydrin (0.25% in acetone) was used to visualize the amino acid spots according to the dip method described by Toennies and Kolb (6). The density of the ninhydrin spots at the glutamate locus was measured with the aid of an Ansco-Sweet densitometer.

Results of a typical experiment showed that both with the vegetative cell and spore extracts, oxalacetate (Fig. 1) and glutamate (Table I) formation increased as incubation progressed. Neither glutamate nor oxalacetate were produced in measurable quantities when reaction systems contained either boiled extract or no extract.

It is of interest to note that whereas Hardwick and Foster (3) reported that 107 μg. of oxalacetate, as measured manometrically, were produced/mg. protein N by *Bacillus mycoides* vegetative cell extracts, we found approximately 660 μg. of oxalacetate being produced/mg. protein N by extracts of *B. megatherium* vegetative cells in the first 30 min. of the reaction.

It is apparent from these results that the spores of *B. megatherium* have nearly as much glutamic–aspartic transaminase as do the vegetative cells. These results, then, cast doubt on the conclusions reached by Hardwick and Foster (3) to the effect that "Enzymes in the vegetative cell are destroyed or lost during sporogenesis."

TABLE I

Glutamate Production via Glutamic–Aspartic Transaminase of B. megatherium *Spore and Vegetative Cell Extracts*

	Density[a] at glutamate locus with extracts of:	
Incubation	Spores	Vegetative cells
min.		
0	0.92	0.98
60	1.11	1.38
240	1.30	1.57

[a] Density is that of the ninhydrin-developed chromatogram spots using the Ansco-Sweet densitometer. The density of the blank paper was 0.72; that of the extract alone, in the same concentration as in the reaction mixtures, was 0.86.

References

1. Levinson, H. S., and Sevag, M. G., *J. Gen. Physiol.* **36**, 617 (1953).
2. Levinson, H. S., and Sevag, M. G., *J. Bacteriol.* **67**, 615 (1954).
3. Hardwick, W. A., and Foster, J. W., *J. Bacteriol.* **65**, 355 (1953).
4. Foster, J. W., and Heiligman, F., *J. Bacteriol.* **57**, 613 (1949).
5. Tonhazy, N. E., White, N. G., and Umbreit, W. W., *Arch. Biochem.* **28**, 36 (1950).
6. Toennies, G., and Kolb, J. J., *Anal. Chem.* **23**, 823 (1951).

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